APPENDIX ONLINE

RESEARCH DESIGN AND METHODS

SUBJECTS:
In this study two different populations were investigated. For methodological and clinical studies (Figs 1 and 2 and Table 1 in the main text) we investigated 764 subjects. They were recruited from the general adult population in the Stockholm area. They came to the laboratory in the morning after fasting overnight. Body weight, waist and hip circumferences were determined and venous blood samples were obtained for determination of plasma glucose, insulin, cholesterol, HDL-cholesterol, and triglycerides as described (1).These clinical measures were not obtained on all the subjects although adipose biopsies were obtained from all subjects. The values for insulin and glucose were used to indirectly evaluate in vivo insulin sensitivity in 719 subjects by the so called HOMA index, which is insulin times glucose divided by 22.5. This index has been proven useful in large scale studies on insulin resistance as discussed (2). On a separate group of 59 subjects (19 men and 40 women, 22-56 years, BMI 18-56 kg m^2, 44 nonobese and 15 obese) we compared HOMA index with direct measurements of *in vivo* insulin sensitivity using the hyperinsulermeric clamp as described (3) by infusing insulin intravenously at a rate of 12 mU/m^2 body surface area x minutes. The relationship between 10log HOMA index and the determination of insulin sensitivity by the clamp was evaluated by regression analyses (Supplementary Fig 1C). The correlation coefficient was 0.83 and $p < 0.0001$.

The study of adipocyte turnover (Fig. 3 in main text) was conducted on a separate group of subjects who had previously been investigated (4). The turnover data were re-calculated and set in relation to adipose tissue morphology as described in the main text. These studies required very large amounts of adipose tissue (>100 g, preferably about 200 g). Such biopsies could not be obtained by usual procedures (policlinical biopsy or removal during routine selective general surgery). Instead we had to use tissue that was a waste product after large cosmetic liposuctions or after abdominal wall reconstruction. In order to obtain such rare samples we used several plastic surgery units in the Stockholm county area.

ADIPOSE TISSUE STUDIES:
Determination of body fat content: It was necessary to utilize the same measure for body fat calculation in the two main studies mentioned above. Therefore we used on all subjects the formula based method, which included values of BMI, sex and age (5), since we could not place machinery equipment on all sites for plastic surgery. In order to evaluate the formula based method for body fat determination in its use for asses adipocyte morphology we also measured body fat by bioimpedance (Body Stat Ltd, Isle of Man, British Isles) on 555 of the subjects in the methodological study. These data were used to compare measures of adipose morphology using formula versus bioimpedance. Both body fat assessments were validated against Dual Energy X-ray Absorbtiometry (DEXA). For this purpose we investigated 16 men and 23 women (BMI 17-46, age 31-79 years, 28 nonobese and 11 obese). As shown in Supplementary Fig. 1A and B there was an excellent correlation between DEXA on the one hand, and body fat measured either with the formula or with bioimpedance, on the other hand. Correlation coefficients were 0.92-0.94, intercepts did not differ significantly from zero and the slopes were near 1 (i.e. 0.93-1.07).

Determination of adipocyte size and number: Isolated fat cells were obtained exactly as described (6). The diameters of 100 cells were measured with a calibrated light microscope. The mean volume and weight of the isolated adipocytes were calculated by provided formulas (7). The total number of fat cells in the body was determined by dividing total body fat content (measured by the formula) with the mean adipocyte weight. There is some variation in mean fat cell weight between different adipose regions. However, as discussed in detail (8), the variations are rather small. In nonobese subjects the estimated total fat cell number is 3.2-3.3 x 10^{10} whether one, two or three adipose regions are used (8).

Models fo the determination of the relationship between adipocyte volume and fat mass, and of adipocyte turnover

Here, we provide a brief summary of the mathematical models used in this study. For a more thorough description of the models, see the supplement of (4).

1. Model describing the relationship between adipocyte average volume and body fat mass

We assume that the number of adipocytes in a human body, n, has a lower limit n_0 which may be the initial number of fat cells from birth. Furthermore, we assume that the number of adipocytes may increase linearly with the body fat mass m through a proportionality constant k_f. The number of adipocytes in the body is thus described by equation 1:

$$n = n_0 + k_f \cdot m \tag{1}$$

The average adipocyte volume, V, is described by equation 2:

$$V = \frac{V_{tot}}{n} = \frac{\frac{m}{g_f}}{n_0 + k_f m} = \frac{\frac{m}{g_f \cdot n_0}}{1 + \frac{k_f}{n_0} m} \tag{2}$$

where V_{tot} is the total body fat volume and g_f is the density of body fat. This equation describes a saturation curve, with two unknown parameters $(g_f \cdot n_0)^{-1}$ and (k_f / n_0) that can be fit to adipocyte volume and body fat mass data. In the case that adipocyte number does not increase with fat mass (i.e. k_f equals zero), the average adipocyte volume increases linearly with fat mass as described by equation 3:

$$V = \frac{m}{g_f \cdot n_0} \tag{3}$$

Average adipocyte volume V is obtained by microscopic determination of adipocyte diameter, and calculated using a formula (7):

$$V = \frac{\pi}{6}(\bar{d}^3 + 3\sigma^2 \bar{d}) \tag{4}$$

where $\bar{d}$ is the average adipocyte diameter and σ^2 is the diameter variance. Finally, fat mass m is obtained through determination of body fat percentage (*BFP*) by measurement with bioimpendance or DEXA, or through subject BMI, sex and age using a well established formula (5):

$$BFP = 1.46 \cdot BMI + 0.12 \cdot AGE - 11.61 \cdot SEX - 10.02 \tag{5}$$

where *SEX* equals 0 or 1 for female or male subjects respectively.

2. Model describing adipocyte turnover rate

In order to model adipocyte turnover, we make the following assumptions. a) At subject age t_0, all adipocytes have age 0, i.e. an initial population of adipocytes are formed at t_0. b) Adipocytes die at constant rate γ. c) After t_0, adipocytes are formed at a constant rate β. We formulate these assumptions as a linear partial differential equation with an age structure,

$$\frac{\partial n(t,a)}{\partial t} + \frac{\partial n(t,a)}{\partial a} = -\gamma n(t,a) \tag{6}$$

where $n(t,a)$ is the population density of adipocytes of age a at subject age t. With initial and boundary conditions $n(t_0,a) = N_0\delta_0(a)$ and $n(t,0) = \beta$, where δ is the Dirac delta function and N_0 is the initial number of adipocytes, we obtain the solution

$$n(t,a) = N_0\delta_{t-t_0}(a)e^{-\gamma a} + \beta e^{-\gamma a} \quad (7)$$

for $0 \le a \le t - t_0$. It follows that, for $t > t_0$, the average adipocyte age $\langle a \rangle$ is described by

$$\langle a \rangle = \frac{\gamma(t-t_0)e_t - b(t-t_0)e_t + \frac{b}{\gamma}(1-e_t)}{\gamma e_t + b(1-e_t)} \quad (8)$$

where $e_t = e^{-\gamma(t-t_0)}$ and $b = \frac{\beta}{N_0}$. Furthermore, the number of adipocytes at age t is given by

$$N(t) = \int_0^{t-t_0} n(t,a)\,da = \frac{\beta}{\gamma}(1 - e^{-\gamma(t-t_0)}) + N_0 e^{-\gamma(t-t_0)} \quad (9)$$

Using a ^{14}C normalization curve K (9), and measuring the average ^{14}C level C in the adipocytes of individual subjects, we can now fit the parameters N_0, β, γ, and t_0:

$$C = \frac{\int_0^{t-t_0} K(a)n(t,a)\,da}{N(t)} \quad (10)$$

However, all parameters cannot be estimated by equation 10 without further assumptions. We use four different scenarios:

Default: Death rate γ and subject age t are large enough to ignore the initial cell population, i.e. N_0 is small and $t_0 = 0$. It follows that only γ is left to estimate, and can be done so individually in each subject.
Scenario A: Same as above, but instead an average γ is calculated for different subgroups in order to enable comparison to results from the scenarios below.
Scenario B: For age $t \ge t_0$, the cell number remains constant; γ and t_0 remains to be estimated in different BMI groups.
Scenario C: For different guesses of t_0, γ and b = β/N_0 are estimated in different BMI groups.

The fits from the above scenarios give us reliable estimates of adipocyte death and birth rates γ and β. In order to estimate N_0 and t_0, we fit equation 9 above using adipocyte number data in a set of subjects of wide age range, where adipocyte number is obtained through use of equations 4 and 5 above.

RESULTS

A clinical evaluation of the adipose tissue morphology was performed in 472 women with complete information on measured clinical variables (Supplementary Table 1). The number of men with complete information was too small for a reliable comparison. Hypertrophy women were slightly (p=0.01) older than hyperplasia women but BMI was not significantly different between the two groups. Those with hypertrophy had significantly larger waist circumference and waist-hip ratio than hyperplasia women. In addition they had higher values for HOMA index and circulating insulin, total cholesterol, and triglycerides in comparison to hyperplasia women and their HDL-cholesterol was lower. As expected hypertrophy women had larger but fewer fat cells than hyperplasia women. Thus, in women adipose hypertrophy associates with a metabolic syndrome like phenotype in comparison with hyperplasia in spite of similar BMI. Similar results were obtained if the women who had chronic disease or were on regular medication (n=48) were excluded (Table not shown).

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Supplementary Table 1. Clinical findings in women with adipose hyperplasia or hypertrophy

Variable	Hyperplasia (n=254)	Hypertrophy (n=218)	P-value
Age, years	38±10	40±11	0.01
Waist, cm	100± 22	105±19	0.01
Waist to hip, ratio	0.895±0.085	0.924±0.098	0.0005
BMI, kg/m^2	32.5±9.4	33.1±8.1	0.37
p-glucose, mmol/l	5.2±1.4	5.4±1.0	0.12
p-insulin, mU/l	10.1±7.8	13.0±7.7	<0.0001
10log HOMA, index	0.25±0.33	0.42±0.29	<0.0001
pl-cholesterol, mmol/l	4.9±1.0	5.1±1.1	0.033
pl-HDL cholesterol, mmol/l	1.40±0.39	1.28±0.36	0.001
pl-triglycerices, mmol/l	1.2±0.8	1.5±0.8	0.002
Fat cell volume, picolitres	555±224	825±209	<0.0001
Fat cell numberx10^{10}	7.9±2.8	5.3±1.7	<0.0001

Values are mean ± SD. Age was compared by unpaired t-test. Since it was slightly different between groups the remaining values were compared by analysis of co-variance with age as co-factor.
pl=fasting plasma.

Supplementary Figure 1. Relationship between formula (a) and bioimpedance (b) derived percentage body fat, and DEXA. 10log HOMA index and glucose uptake during hyperinsulinemic euglycemic clamp (M/1=mg glucose • kg body weight-1 x min -1) (c). Linear regression analysis was performed.